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PRINCIPAL INVESTIGATOR: David Stern, Ph.D.

CONTRACTING ORGANIZATION: Yale University

New Haven, Connecticut 06510

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13. ABSTRACT (Maximum 200 Words)

Breast cancer is one of the leading causes of cancer deaths of women in the United States. Fortunately, this disease is no longer a "black box" that can only be studied empirically. Rather, recent advances in understanding of normal mammary development and carcinogenic processes have identified a number of specific genes and processes that are dysregulated in breast cancer. This means that research on breast cancer has finally advanced to the stage where a concentrated effort in translational research will yield great strides in detection, diagnosis, and treatment. The Molecular Medicine graduate training program at Yale was recently developed to address these issues. This program was developed to offer an interdisciplinary course of study that will foster an integrated view of disease, built upon a rigorous foundation of basic sciences. The emphasis on disease mechanisms and translational research is unique to Molecular Medicine, and distinguishes it from other pre-doctoral programs at Yale. The Predoctoral Training Program in Breast Cancer Research will recruit individuals interested in careers in breast cancer research to the Molecular Medicine Program, provide specialist training in breast cancer-specific areas, and integrate their training experience with basic scientists and clinicians investigating breast cancer at Yale.

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Table of Contents

Cover	1
SF 298	2
Table of Contents	3
Introduction	4
Body	4
Key Research Accomplishments	12
Reportable Outcomes	12
Appendicesrecruitment poster	13

(4) INTRODUCTION

The past decade has witnessed a revolution in the power of biologists to investigate fundamental mechanisms underlying disease. This change has resulted from major advances in biological research, coupled with the extraordinary power of modern molecular genetics for identification of gene mutations in disease. The result is that investigation of many human diseases has gone beyond the descriptive level to the root causes. This new knowledge means that the tools of genetics, immunology, cell biology, molecular biology, and other disciplines can now be combined to investigate disease pathogenesis, and to apply these findings to issues of diagnosis and treatment. The Molecular Medicine graduate training program at Yale was recently developed to address these issues. This program was developed to offer an interdisciplinary course of study that will foster an integrated view of disease, built upon a rigorous foundation of basic sciences. The emphasis on disease mechanisms and translational research is unique to Molecular Medicine, and distinguishes it from other pre-doctoral programs at Yale. The Predoctoral Training Program in Breast Cancer Research will recruit individuals interested in careers in breast cancer research to the Molecular Medicine Program, provide specialist training in breast cancer-specific areas, and integrate their training experience with basic scientists and clinicians investigating breast cancer at Yale.

(5) BODY

We are now completing the first year of this new training program.

Recruitment. Class entering Fall 1999. As discussed in the proposal, trainees are recruited through the Pharmacological Sciences and Molecular Medicine Program of the university-wide combined BBS training program. The award for this grant was activated in summer, 1999 to fund students entering the Program in Fall, 1999. Because of the timing of the USAMRMC grant program relative to the academic year, students funded during year 1 of the grant had been admitted prior to approval of the grant. These students had been admitted the previous Spring. A description of the applicant and admitted pool is provided in Table 1. Students chosen for funding by the program in the first year were those whom we felt were most likely to remain in cancer research from the entering class.

<u>Class entering Fall, 2000.</u> In order to enhance recruitment for the program, we assembled a poster and a Web site (http://info.med.yale.edu/pathol/bcr/index.html) as adjuncts to the BBS recruiting information. A copy of the poster is provided in the Appendix. This poster was distributed by mail to over 3500 academic units including programs in Biological and Biomedical Sciences, Developmental Biology, Experimental Biology, Genetics, and other relevant disciplines.

Training. The first year in graduate school consists of course work and a series of research rotations. All incoming students were oriented along with other students in the Pharmacological Sciences and Molecular Medicine Track, and assigned to trainers and Track Directors William Sessa and David Stern as advisors. The advisors met with the students to jointly plan out a curriculum for the first year. In year 1, this consists of course work and three laboratory rotations. The rotations serve to train individuals in design, execution, and interpretation of laboratory research projects, to expose the trainees to a variety of research experiences, and to enable trainees to identify compatible

dissertation advisors. Students are required to choose a dissertation advisor the end of the first year.

Course work. As discussed in the proposal, the purpose of coursework is to ensure that students have a strong basic science foundation that complements undergraduate coursework, and to provide students with advanced and specialty knowledge. This process will continue in year 2, with inclusion of breast-cancer-specific training. Classes taken by the first year students are described in Table 3, and include "foundation" courses such as cell biology and genetics, and more specialized coursework in cancer and disease mechanisms.

<u>Year 1, rotations.</u> The rotation advisors and rotation topics for year one are shown in Table 2. Four of the 15 rotations were done in labs of trainers, and each student except KPK rotated in at least two trainers' labs. (KPK did settle in a trainer lab.)

A description of rotation projects follows.

Kian Peng Koh.

Barbara Ehrlich

Localization of the low-affinity IP3 binding site on the IP3 receptor.

Yung Chi Cheng

Characterization of the phosphotransferase reaction intermediates of the nucleoside-diphosphate kinases Nm23-H1 and Nm23-H2.

Jordan Pober

Characterization of the anti-apoptotic pathways activated by the

TNF/PI3-kinase/Akt pathway in endothelial cells.

Soo Jung Lee.

David Stern

Rad53 binding proteins induced by the DNA replication checkpoint

Willam Sessa

Cellular localization of phosphorylated endothelial nitric oxide synthase

Michael Snyder

Analysis of protein kinases using protein microarrays.

Jessica Hawes.

Archibald Perkins

Protocol Refinement/Development of a cDNA microarray assay for comparison of cancerous and non-cancerous tissues.

David Rimm

Search for proteins that preferentially bind tyrosine-phosphorylated beta-catenin, which may be involved in the differential regulation of beta-catenin between its roles in cell-cell adhesion and regulation of the TCF/LEF transcription complex.

Joseph Madri

Examined the cellular localization of MMP 2, MT1-MMP, and TIMP 2 at sites of invadopodia during angiogenesis.

Craig Ĉrews

Investigated Methionine Aminopeptidase 2 activity as a necessary factor in myristoylation of c-Abl and subsequent stabilization/activation of p53

Julie Wu.

Lucia Languino.

Beta1a and Beta1c integrin regulation of focal adhesion kinase

David Stern

Search for mutations in the candidate tumor suppressor gene CHK2 in breast cancer tissue

Anton Bennett.

SHP-2 regulation of p38 MAPK.

Selection of dissertation advisers. Since, under the BBS program, students are free to rotate throughout the university, it was inevitable that there would be some attrition between years one and two. Of the three students funded in year one of the program, two (KPK and SL) affiliated with program trainers. A third (MG) affiliated with Dr. Henrik Dohlman, an expert on G-protein coupled receptors. In deciding whether these students would be reappointed to the program in year 2, we took into account, not only the adviser, but the relatedness of the dissertation project to breast cancer. On this basis, we retained Soo-Jung Lee in the program. Mr. Koh will be working in trainer Pober's laboratory, but in an area only distantly related to cancer. Ms. Granovskaya will work on sorting of caveolar proteins using a yeast system. Since caveolae are implicated as depots for a number of important growth regulatory signaling proteins, the project has strong cancer relevance, but Dr. Dohlmann is not one of our trainers.

In year 2 these students will complete coursework, including breast cancer training, qualifying exams, and will begin to lay the foundation for the dissertation. Although the students have dissertation advisors, the dissertation project is not formalized until the prospectus exam, which must be completed by the end of year 3. A brief description of research directions of the five students is now provided, with the caveat that it may be some time before the projects fully evolve.

Trainees and their projects.

Marina Granovskaya (no longer funded by training program). Ms. Granovskaya is not presently funded by this program because her dissertation adviser, Henrik Dohlman, is not a trainer on this grant. Marina Granovskaya's current project is to (1) reconstitute caveolae formation in yeast S. cerevisiae (an organism that lacks caveolin or caveolae), (2) determine the functional consequences of caveolae formation for G protein signaling in yeast, (3) isolate G protein mutants that fail to assemble into caveolae, and (4) determine the functional consequences for analogous G protein mutants in mammalian cells.

Breast cancer relevance. Caveolae are thought to function as functional domains within the plasma membrane where specific subsets of signaling proteins gather. Several proteins important to breast cancer have been localized to caveolae, including oncogenes HER2/neu and Src. Shuttling in and out of caveolar compartments is thought to regulate interactions of intramembrane signaling molecules.

Kian Peng Koh (no longer funded by training program). Mr. Koh is in the laboratory of trainer Jordan Pober. However, since he is working on the cardiovascular system, his work falls outside the scope of the training grant, so he is being funded through other resources. His subject is the mechanism of endothelial dysfunction in the pathogenesis of transplantation-associated arteriosclerosis. The immediate goal is to assess the possibility of performing vascular functional assays on arteries cultured for 1-2 weeks on a collagen gel system. If

feasible, the system will provide a novel in vitro/ex vivo approach to study whether leukocytes, cytokines or the combination of factors induces endothelial dysfunction in transplanted vessels.

Breast cancer relevance is limited, although the process angiogenesis is important in carcinogenesis.

<u>Soo-Jung Lee.</u> Ms. Lee is working in the laboratory of the program director, Dr. Stern. The major components of DNA checkpoint pathways have been shown to be conserved between budding yeast and humans. The Stern lab is investigating DNA checkpoint pathways connecting DNA damage to activation of the yeast ortholog of human Atm (yeast Mec1), which in turn activates human Chk2 (yeast Rad53). Work in Dr. Stern's lab has developed an explicit model for the mechanism, which involves an intermediary protein possibly homologous to Brca1 (budding yeast Rad9) interacting via a phospho-peptide recognition domain on Rad53. Ms. Lee is determining how complexes containing yeast proteins homologous to human Hus1, Rad1, and Rad9 (unrelated to yeast Rad9) regulate this pathway.

Breast cancer relevance. This work is directly relevant to breast cancer, since it is now clear that Chk2 is an intermediary linking DNA checkpoint pathways from candidate breast cancer tumor suppressor Atm to breast cancer tumor suppressor p53; since Chk2 phosphorylates and modulates Brca1 function, and since Chk2 mutations are found in variant p53+ forms of Li-Fraumeni syndrome, which predisposes to breast cancer and other cancers.

Julie Wu. Ms. Wu is working in the laboratory of trainer Anton Bennett. Mitogen activated protein kinase (MAP) phosphatase 1 (MKP1) is a dual specificity tyrosine/threonine phosphatase that is highly regulated by growth regulators and stress. It dephosphorylates MAPK family members. MKP1 regulates the activities of p38 (Stress activated protein kinase) and ERKs. Attenuation of MAPK activity after activation is a tightly regulated process.

The four isoforms of p38 are each encoded by distinct genes. They response to different stimuli and they differentially regualte biological functions including apoptosis, proiferation and differentiation. Ms. Wu will examine MKP1 regulation of p38 in the liver during the stress response, and will also evaluate p38 activity in MKP1 knockout mice.

Breast cancer relevance. MAPKs are major regulators of cell growth and apoptosis. Oncogenes such as Ras and ErbB2 function in part through activation of MAPKs. Regulation of MAPK attenuation may be as important as regulation of activation. Inability to attenuate the activity of activated proteins results in abnormal biological processes. Inability to attenuate a p38 MAPK activity during stress may uncouple the cell's ability to respond to stress and repair induced damage. This may result in gene mutations, and/or unregulated growth, two hallmarks of cancer.

Table 1. Applicant pool. Class entering 1999-2000.

INSTITUTION	DEGREE	YEAR	GPA	V	Q	A	admitted	enrolled
Pitzer College	BA	1998	3.65	560	680	750	Y	Y
Mass Institute of Technology	BS	1999	£	690	t	<u>}</u>	Y	Y
Connecticut, Univ ofStorrs	BA	1996		570	B	§	Ÿ	Y
Moscow State University	BS/MS	1999		5	610	}	Y	Y
Weber State University	BS	1999	3.57	<u> </u>	740	{	Y	Ÿ
Williams College	BA	1999	ł	650	!	§	Y	Y
Yale University	BS	1995		3	740	ŧ.	Y	Y
Seoul National University	BS	1994	3.15	550	ŝ	ł	Y	Y
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				660	770	670		
Hollins College	BA	1997	3.95	650	¥ .	ş.	Y	Y
Florida State University	BS	1994		ŧ	580	3	Y	Y
SUNY At Stony Brook	BS	1999	3.60	410	8	ž.	Y	Y
Swarthmore College	BA	1998		710	690	750	Y	
Cornell University	BA	1999	4.00	620	800	580	Y	
Calif, Univ of-Davis	BS	1998	3.69	600	780	660	Y	
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CUNIX A4 C4 D1	D.C.	1007	2.64		800			
SUNY At Stony Brook	BS	1997	3.64	1	710	£	Y	***************************************
Rutgers UnivNew Brunswick	BA	1994	3.22	1570	650	640	Y	
				610	720	780		÷
Lycoming College	BS	1998	ļ		800		Y	
Univ of Maryland-CollegePark	BS	1999			780	£	Y	
Yale University	BS	1999			750	3	Y	
Cornell University	BA	1995	3.74	i	720	I	Y	
Michigan, Univ ofAnn Arbor	BS	1997	3.83	•	790	}	Y	***************************************
Central Michigan University	BS	1999	3.98	3	740	1	Y	
Wisconsin, Univ ofMadison	BS	1997	3.37	3	640	£	Y	······································
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					650			
Willamette University	BA	1999	3.02	510	700	770	Y	
Arizona, University of	BS	1999					Y	
London, University of	BS	1999		3	740	٤	3	
Humboldt State University	BS	1999	3.74	460	t	3		
Carnegie Mellon University	BS			3	730	•	3	
Albany College of Pharmacy	BS	1999	3.60	490				
Harvard-Radcliffe College	BA	1999			750	,	1	
Johns Hopkins University	MPH	1994		620	670	590		
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Notice of York Mine II-	MD	1005	ļ		720			
National Yang Ming University	MD	1995		3	790	£	3	
Other Institution	BS	1993	2.02	£	770	3	1	
Virginia, University of	BS	1997	3.03	540	B	3	1	
Shanghai Medical University	BS	1998		3	780	£	3	
Other Institution	MS	1995	2.06	3	790	£	§	
American Univ Beirut	BS	1995	2.96	ŧ	:	}	ŧ	
Shanghai Medical University	BS	1996	<u></u>	10/0	790	1/00	<u></u>	

George Mason University	BS	1998	3.75	540	590	630	······································	T
Wesleyan University (Conn)	BA	1998		520	550	530		
				,	,	,		
	- 50	1000	2.25	540	680	570	***************************************	
Calgary, University of	BS	1999	3.35	570				
Northeastern University	MS	1999		• •	750			ļ
Other Institution	BS	1993		1	710		······	
Pace UniversityPleasantvill	BS	1999	•	570				
Howard University	BS	1998	3.16	320	400	360		
				200	, 410	200		
Habrary University	DVM	1992			670		***************************************	
Hebrew University Yale University	BA	1992	3.30	670	3	. 1	······································	
Tale University	DA	1997	3.30	070	020	070		
				700	, 660	, 680		
William & Mary, College of	BS	1999	2.87	600				
Earlham College	BA	1999	§	500	į.	: :		-
National Taiwan University	MD	1997		1	800	i		-
Nebraska, Univ ofLincoln	BS	1999	3.96	630	680	800	***************************************	
Calif, Univ of-Berkeley	BA	1998	3.52	580	750	610		
McMaster University	BS	1998		1	740	3 8		
Pittsburgh, Univ ofPittsburg	BS	1999	3.91	450	710	480	***************************************	-
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						500		
London, University of	BS	1999			640	3		
New Mexico State ULas Cruce	BS	1997	3.91	400	490	560		
				120	600	700		
Imperial College	BS	1999				560		
Rochester, University of	MS	1995		3	8	500		
Calif, Univ of-Berkeley	BS	1998	3.31	ŧ	:	550		
Other Institution	MD	1999	3.59	ŧ	1	730		
Other Institution	BS	1996	3.37	3	1	700		
Hampshire College	BA	1999			1	630		
Other Institution	MD	1998		1000	1720	050		
Tufts University	DVM	1993		460	670	540		
Dartmouth College	BA	1998	3.21	§	L	710	1	
Wooster, College of	BA	1999	3.63					
Centre College (Kentucky)	BS	1999	3.31			570		
McGill University	BS	1997	3.48	3	i	670	3	
Fudan University	BS	1999	13.10	•		670	ŧ	
Oklahoma State University	MS	1991	 	3		670	3	
Other Institution	MD	1998	-	ŧ	:	720	Į.	
Other Institution	BS	1999	3.95	3		1	ŧ	
Calif, Univ of-Berkeley	BA	1998	3.65					
Cam, Only of Borkerey	<i>D</i> 1.	1770	3.03	1.0	1,,,	1.		
				580		710		
Trinity University (Texas)	BS	1999	3.21	640	620	730	1	
Oklahoma, University of	BS	1998	3.68	560	750	690		
Gannon University	BS	1995	3.05	1	1	1	1	

Calif, Univ of-Berkeley	BS	1999	3.67	T	***************************************	***************************************
Tokyo, University of	BE	1999				<u> </u>
Rochester Inst of Technology	BS	1999	3.89		***************************************	*
Bennett College	BS	1999	3.92		***************************************	·
Nankai University	BS	1997				†
SUNY At Stony Brook	BS	1999	3.32		······································	
National Taiwan University	BS	1996				·
Beijing University	BS	1999			***************************************	
Beijing University	BS	1999			***************************************	
				 1	***************************************	

Table 2. Students and rotations.

	students entering Fall, 1999 Student	Rotations and <u>Dissertation</u> <u>Advisors</u> *= BCRTP trainer	students entering Fall, 2000
Year 1 1999-2000	Marina Granovskaya Soo-Jung Lee Kian Peng Koh	Perkins*, Languino*, <u>Dohlmann</u> <u>Stern</u> *, Sessa*, Snyder Ehrlich, <u>Pober*,</u> Cheng	
Year 2 2000-2001	Jessica Hawes Soo-Jung Lee Julie Wu	Perkins*, <u>Crews</u> *, Madri* <u>Stern*</u> , Sessa*, Snyder Languino*, <u>Bennett</u> *, Stern*	Seda Eminaga Kristen Massimine Alexander Urban

Table 3. Coursework by trainees, year 1.

	Granov -skaya	Hawes	Lee	Koh	Wu
Fall term.					
Cell Biology 602a		•	•	•	•
Molecular Cell Biology					
Genetics 625a		•	•	•	
Basic Concepts of Genetic					
Analysis					
Pathology 680a seminar	•	•	•	•	•
Topics in Molecular					
Medicine: Matrix Biology			·		
Pharmacology I:Maintaining	•				•
and restoring homeostasis					
Pathology 640a	•				
From Molecular Biology to					
Molecular Medicine			ſ		
MBandB 752a	•				
Genomics/Bioinformatics					
Spring term.					
Pathology 650b	•	•	•		•
Cellular and Molecular					
Biology of Cancer					
Pathology 690b		•		•	
Mechanisms of Disease					
Pharmacology II: Interfering	•	•	•	•	•
selectively			***************************************		
Pharm 502b seminar	•	•	•	•	•

(7)Accomplishments

- Recruitment of a highly qualified group of students interested in cancer research.
- Guiding students through an appropriate series of classes, including training in cell biology, disease mechanisms, cancer, and pharmacology.
- Providing students with research rotations relevant to cancer research.
- Selection of second year students with dissertation projects relevant to breast cancer research for further training through the program.

Reportable outcomes.

None.

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Breast cancer is one of the leading cancer killers of women. Thanks to recent scientific advances, this disease is finally beginning to yield ground. The Training Program in Breast Cancer Research offers an interdisciplinary course of studies leading to the

the training:

The program fosters an integrated view of disease, built upon a rigorous foundation of basic sciences including cell biology, molecular genetics, and

the faculty:

David F. Stern, PhD, Director Dario C. Altieri, MD,PhD Karen S. Anderson, PhD Anton. M. Bennett, PhD

leading cancer killers of women. Thanks to recent scientific advances, this

disease is finally beginning to yield ground. The

Training Program in Breast

interdisciplinary course Cancer Research offers an

of studies leading to the Ph.D. Unique features of

this program are the

emphasis on disease mechanisms, and translation

to the clinic, as well as the

specific focus on breast

cancer. Areas of dissertation

research include signal

basic sciences through course

gain specialty training in work and seminars. Students

breast cancer through joint activities with the Yale Comprehensive Cancer

transduction, cell cycle

regulation, apoptosis,

mammary development,

cancer genetics, and

pharmacology.

is a component of the Center. The training program

university-wide combined

program in Biomedical and

Biological Sciences (BBS).

the training:

the faculty.

The program fosters an

integrated view of disease,

built upon a rigorous

foundation of basic sciences

David F. Stern, PhD, Director

Dario C. Altieri, MD, PhD Karen S. Anderson, PhD

Anton. M. Bennett, PhD

including cell biology,

molecular genetics, and

pathogenesis. Students

acquire a firm foundation in

Nancy Berliner, MD Jose Costa, MD

Craig Crews, PhD

Pietro DeCamilli, MD

Michael DiGiovanna, MD, PhD

Daniel C. DiMaio, MD, PhD

Xin-Yuan Fu, MD, PhD

James Jamieson, MD, PhD

Richard Lifton, MD, PhD Lucia Languino, PhD

Joseph Madri, MD, PhD

Vincent Marchesi, MD, PhD

Jon Morrow, MD, PhD

Archibald Perkins, MD, PhD

Jordan Pober, MD, PhD Michael Reiss, MD David Rimm, MD, PhD

William Sessa, MD, PhD Mark Solomon, PhD

Hong Sun, PhD

2

Barbara Ward, MD

For more information:

emphasis on disease mechanisms, and translation to the clinic, as well as the specific focus on breast cancer. Areas of dissertation research include signal transduction, cell cycle regulation, apoptosis, mammary development, cancer genetics, and pharmacology.

basic sciences through course work and seminars. Students gain specialty training in breast cancer through joint activities with the Yale Comprehensive Cancer Center. The training program is a component of the university-wide combined program in Biomedical and Biological Sciences (BBS).

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For more information:

http://info.med.yale.edu/pathol/bcr

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students may elect to train with other BBS faculty at Yale